

1: Genome Res 1996 Sep;6(9):791-806

Normalization and subtraction: two approaches to facilitate gene discovery.

Bonaldo MF, Lennon G, Soares MB.

Department of Psychiatry, College of Physicians and Surgeons of Columbia University, New York, New York, USA.

Large-scale sequencing of cDNAs randomly picked from libraries has proven to be a very powerful approach to discover [putatively] expressed sequences that, in turn, once mapped, may greatly expedite the process involved in the identification and cloning of human disease genes. However, the integrity of the data and the pace at which novel sequences can be identified depends to a great extent on the cDNA libraries that are used. Because altogether, in a typical cell, the mRNAs of the prevalent and intermediate frequency classes comprise as much as 50-65% of the total mRNA mass, but represent no more than 1000-2000 different mRNAs, redundant identification of mRNAs of these two frequency classes is destined to become overwhelming relatively early in any such random gene discovery programs, thus seriously compromising their cost-effectiveness. With the goal of facilitating such efforts, previously we developed a method to construct directionally cloned normalized cDNA libraries and applied it to generate infant brain (INIB) and fetal liver/spleen (INFLS) libraries, from which a total of 45,192 and 36,088 expressed sequence tags, respectively, have been derived. While improving the representation of the longest cDNAs in our libraries, we developed three additional methods to normalize cDNA libraries and generated over 35 libraries, most of which have been contributed to our Integrated Molecular Analysis of Genomes and Their Expression (IMAGE) Consortium and thus distributed widely and used for sequencing and mapping. In an attempt to facilitate the process of gene discovery further, we have also developed a subtractive hybridization approach designed specifically to eliminate (or reduce significantly the representation of) large pools of arrayed and (mostly) sequenced clones from normalized libraries yet to be (or just partly) surveyed. Here we present a detailed description and a comparative analysis of four methods that we developed and used to generate normalize cDNA libraries from human (15), mouse (3), rat (2), as well as the parasite *Schistosoma mansoni* (1). In addition, we describe the construction and preliminary characterization of a subtracted liver/spleen library (INFLS-SI) that resulted from the elimination (or reduction of representation) of ~5000 INFLS-IMAGE clones from the INFLS library.

PMID: 8889548 [PubMed - indexed for MEDLINE]

IDENTIFIERS

dbEST Id: 7072761
 EST name: UI-R-Y0-abg-a-03-0-UI.r1
 GenBank Acc: BF543337
 GenBank gi: 11634444

CLONE INFO

Clone Id: UI-R-Y0-abg-a-03-0-UI (5')
 Source: The University of Iowa Program for Rat Gene Discovery and Mapping (Val Sheffield, Bento Soares and Tom Casavant)
 Id as DNA: UI-R-Y0-abg-a-03-0-UI
 Id in host: UI-R-Y0-abg-a-03.r1
 DNA type: cDNA

PRIMERS

Sequencing: M13 Forward
 PolyA Tail: Unknown

SEQUENCE

CAGGAGGCTGATGTTCTCTCTCTTCACAGTTCTCTTCCAGTCCAGACATCTTCGCAAAGAATA
 GGAGGCTCTCTCCCCACAGGTTCCGGGAGGAGTCACTCCAGTCTCTCCCCAGGCATCCAGTT
 CCCATCTTGTCTCTCATCGGAGGAGCATTCTTTGAGGTCTGATCATCATAGCGGTATGT
 GAGGAACTGTTCCCCCTGTGTTATCTTCAGAAAGCTTCTCTGAGGAGAGAGTGGGAACTC
 AGGAAGGAAGTAGGCTCCAGGAGACTTCTCTGCTGTGGCTGAGGAGAGCCAGAGACTGT
 AAAGATCATTTCTGTGAGGAGCCAGCCAGGATCTCTTCCAGAAAAGCATGGGGTCCCAATAT
 TCTAGGAGTCTCTCTCCAGCCATCTCTGGGGAGACTGGTCTCTGGAT

Entry Created: Dec 11 2000

Last Updated: Dec 11 2000

COMMENTS

cDNA Library Preparation: M.B. Soares Lab Clone
 distribution: clones will be available through Research
 Genetics (www.resgen.com). This clone is also available
 through the I.M.A.G.E. Consortium at LLNL
 (info@image.llnl.gov). IMAGE ID= 1790944

LIBRARY

dbEST lib id: 1387
 Lib Name: UI-R-Y0
 Organism: Rattus norvegicus
 Strain: Sprague-Dawley
 Develop. stage: adult
 Lab host: LH10E (Life Technologies)
 Vector: pT7T3D-Pac (Pharmacia) with a modified polylinker
 R. Site 1: Not I
 R. Site 2: Eco RI
 Description: The UI-R-Y0 library is a subtracted library derived from an individually-tagged normalized whole-eye (minus the lens) library. The driver for the subtraction consisted of a pool of all previous libraries (UI-R-A0, UI-R-A1, UI-R-E0, UI-R-E1, UI-R-C0, and UI-R-C1). The tag is a string of 3-5 nucleotides present between the Not I site and the cligo-dT track which allows identification of the library of origin of a clone within the mixture. The subtracted library (UI-R-Y0) was constructed as follows: PCR amplified cDNA inserts from previous library clones from which 3' ESTs had been derived were used as a driver in a hybridization with the normalized whole-eye library in the form of

single-stranded circles. The remaining single-stranded circles subtracted library was purified by hydroxyapatite column chromatography, converted to double-stranded circles and electroporated into DH10B bacteria (Life Technologies) to generate the UI-R-YC library. This procedure has been previously described (Bonaldo, Lennon and Soares, Genome Research 6: 791-806, 1996).

SUBMITTER

Name: Soares, MB
Lab: Program for Rat Gene Discovery and Mapping
Institution: University of Iowa
Address: 451 Eckstein Medical Research Building Iowa City, IA 52242,
USA
Tel: 319 335 8250
Fax: 319 335 9565
E-mail: msoares@blue.weeg.uiowa.edu

CITATIONS

Medline UID: 97044477
Title: Normalization and subtraction: two approaches to facilitate gene discovery
Authors: Bonaldo, M.F., Lennon, G., Soares, M.B.
Citation: Genome Res. 6 (9): 791-806 1996

MAP DATA

||